REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
10-01-2015	Final Report	1-Jul-2009 - 31-Aug-2014

4. TITLE AND SUBTITLE
Mechanisms of Bacterial Spore Germination and its Heterogeneity

5a. CONTRACT NUMBER
W911NF-09-1-0286

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS
Peter Setlow

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Connecticut - Health Center
263 Farmington Ave.

Farmington, CT 06032 -1956

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
56140-MA-MUR.82

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
Great progress has been made in understanding the mechanisms of Bacillus and Clostridium spore germination and its heterogeneity in the 5+ years of the MURI award. The following advances have been made: 1) high resolution structures of four germination proteins have been determined; 2) reasons for the heterogeneity in rates of spore germination have been determined, a major one being variations in levels of germinant receptors (GRs) between individual spores; 3) many factors modulating GR levels in spores have been identified; 4) a number of new early events in germination have been identified including acquisition of spore memory of germinant exposure, and a

15. SUBJECT TERMS
Bacillus, Clostridium, spores, spore germination, germinant receptors, germination heterogeneity

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Peter Setlow
UU	UU	UU	UU		19b. TELEPHONE NUMBER 860-679-2607

Report Title

Mechanisms of Bacterial Spore Germination and its Heterogeneity

ABSTRACT

Great progress has been made in understanding the mechanisms of Bacillus and Clostridium spore germination and its heterogeneity in the 5+ years of the MURI award. The following advances have been made: 1) high resolution structures of four germination proteins have been determined; 2) reasons for the heterogeneity in rates of spore germination have been determined, a major one being variations in levels of germinant receptors (GRs) between individual spores; 3) many factors modulating GR levels in spores have been identified; 4) a number of new early events in germination have been identified including acquisition of spore memory of germinant exposure, and a drastic change in spore inner membrane properties; 5) GRs in B. subtilis spores were shown to assemble in a single focus in spores' inner membrane in a GerD protein dependent manner; 6) large numbers of refinements have been made in technology to simultaneously examine the germination of 100s of individual spores; 7) high-pressure germination of multiple individual spores has been examined; 8) a mathematical model describing spore germination has been developed; 9) much of the work above has been extended to Clostridium spores; and 10) ~90 research papers have or will be published.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
02/06/2015	80.00	Sonali Ghosh, George Korza, Mark Maciejewski, Peter Setlow. Analysis of metabolism in dormant spores of Bacillus species by 31P-NMR of low molecular weight compounds, Journal of Bacteriology, (03 2015): 992. doi:
02/11/2011	9.00	D. Paredes-Sabja, P. Setlow, M. R. Sarker. Germination of spores of Bacillales and Clostridiales species: mechanisms and proteins involved, trends in microbiology, (02 2011): . doi:
02/11/2011	12.00	D. Paredes-Sabja, M. R. Sarker. Effect of the cortex-lytic enzyme SleC from non-food-borne Clostridium perfringens on the germination properties of SleC-lacking spores of a food poisoning isolate, Canadian Journal of Microbiology, (02 2011): . doi:
02/11/2011	10.00	L. Kong, P. Zhang, J. Yu, P. Setlow, Y.Q. Li. Monitoring the kinetics of uptake of a nucleic acid dye during the germination of single spores of Bacillus subtilis, Analytical Chemistry, (02 2011): . doi:
02/11/2011	11.00	P. Zhang, L. Kong, G. Wang, P. Setlow, Y.Q. Li. Combination of Raman tweezers and quantitative differential interference contrast microscopy for measurement of dynamics and heterogeneity during the germination of individual bacterial spores, Journal of Biomedical Optics, (10 2010): . doi:
02/19/2014	58.00	B. Hao, P. Setlow, M.R. Sarker, D. Paredes-Sabja, G. Korza, Y. Li, S. Banawas. The Clostridium germinant receptor protein, GerKC, is located in the spore inner membrane and is crucial for spore germination, Journal of Bacteriology, (11 2013): 5084. doi:
02/19/2014	52.00	Peter Setlow, Jing Yu, Barbara Setlow, Yong-qing Li. Analysis of the germination kinetics of individual Bacillus subtilis spores treated with hydrogen peroxide or sodium hypochlorite, Letters in Applied Microbiology, (10 2013): 259. doi:
02/19/2014	59.00	T, Zhou, Z. Dong, P. Setlow, Y.Q. Li. Kinetics of germination of individual spores of Geobacillus stearothermpohilus as measured by Raman spectroscopy and differential interference microscopy, PLoS ONE, (09 2013): 27497. doi:
02/19/2014	60.00	R. Moeller, M. Raguse, G. Reitz, R. Okayasu, Z. Li, S. Klein, P. Setlow, W. L. Nicholson. Resistance of Bacillus subtilis Spore DNA to Lethal Ionizing Radiation Damage Relies Primarily on Spore Core Components and DNA Repair, with Minor Effects of Oxygen Radical Detoxification, Applied and Environmental Microbiology, (10 2014): 104. doi: 10.1128/AEM.03136-13
02/19/2014	61.00	P. Setlow. Summer meeting 2013 - when the sleepers wake: the germination of spores of, Journal of Applied Microbiology, (12 2013): 1251. doi: 10.1111/jam.12343
02/19/2014	62.00	Barbara Setlow, Shuji Kaieda, Peter Setlow, Bertil Halle. Mobility of Core Water in Bacillus subtilis Spores by 2H NMR, Biophysical Journal, (11 2013): 2016. doi: 10.1016/j.bpj.2013.09.022

02/20/2014 63.00 S. S. Campos, J. R. Ibarra-Rodriguez, R. C. Barajas-Ornelas, F. H. Ramirez-Guadiana, A. Obregon-Herrera, P. Setlow, M. Pedraza-Reyes. Interaction of Apurinic/Apyrimidinic Endonucleases Nfo and ExoA with the DNA Integrity Scanning Protein DisA in the Processing of Oxidative DNA Damage during Bacillus subtilis Spore Outgrowth,
Journal of Bacteriology, (11 2013): 568. doi: 10.1128/JB.01259-13

02/20/2014 68.00 L. Kong, C. J. Doona, P. Setlow, Y.-q. Li. Monitoring Rates and Heterogeneity of High-Pressure Germination of Bacillus Spores by Phase-Contrast Microscopy of Individual Spores,
Applied and Environmental Microbiology, (01 2014): 345. doi: 10.1128/AEM.03043-13

02/20/2014 67.00 J. Li, M. Ma, M. R. Sarker, B. A. McClane. CodY Is a Global Regulator of Virulence-Associated Properties for Clostridium perfringens Type D Strain CN3718,
mBio, (10 2013): 770. doi: 10.1128/mBio.00770-13

02/20/2014 66.00 Marjorie Pizarro-Guajardo, Valeria Olguín-Araneda, Jonathan Barra-Carrasco, Christian Brito-Silva, Mahfuzur R. Sarker, Daniel Paredes-Sabja. Characterization of the collagen-like exosporium protein, BclA1, of Clostridium difficile spores,
Anaerobe, (02 2014): 18. doi: 10.1016/j.anaerobe.2013.11.003

02/20/2014 64.00 K. Nagler, P. Setlow, Y.-Q. Li, R. Moeller. High Salinity Alters the Germination Behavior of Bacillus subtilis Spores with Nutrient and Nonnutrient Germinants,
Applied and Environmental Microbiology, (12 2013): 1314. doi: 10.1128/AEM.03293-13

03/11/2013 48.00 George Korza, Peter Setlow. Topology and accessibility of germination proteins in the Bacillus subtilis spore inner membrane,
Journal of Bacteriology, (04 2013): 1484. doi:

03/17/2014 69.00 Peter Setlow. The germination of spores of Bacillus species: what we know and don't know,
Journal of Bacteriology, (04 2014): 1297. doi:

03/21/2013 47.00 A. Perez-Valdespino, S. Ghosh, E.P. Cammett, L. Kong, Y.-q. Li, P. Setlow. Isolation and characterization of Bacillus subtilis spores that are superdormant for germination with dodecylamine or Ca(2+)-dipicolinic acid ,
Journal of Applied Microbiology, (04 2013): 1109. doi: 10.1111/jam.12125

03/23/2012 34.00 A. Ramirez-Peralta, P. Zhang, Y.-q. Li, P. Setlow. Effects of Sporulation Conditions on the Germination and Germination Protein Levels of Spores of Bacillus subtilis,
Applied and Environmental Microbiology, (02 2012): 0. doi: 10.1128/AEM.07908-11

03/23/2012 35.00 P. Zhang, L. Kong, G. Wang, M. Scotland, S. Ghosh, B. Setlow, P. Setlow, Y.-Q. Li. Analysis of the slow germination of multiple individual superdormant Bacillus subtilis spores using multifocus Raman microspectroscopy and differential interference contrast microscopy,
Journal of Applied Microbiology, (03 2012): 0. doi: 10.1111/j.1365-2672.2011.05230.x

04/02/2012 36.00 Y. Li, A. Davis, G. Korza, P. Zhang, Y.-q. Li, B. Setlow, P. Setlow, B. Hao. Role of a SpoVA Protein in Dipicolinic Acid Uptake into Developing Spores of Bacillus subtilis,
Journal of Bacteriology, (04 2012): 0. doi: 10.1128/JB.00062-12

04/07/2014 70.00 Stephanie Luu, Peter Setlow. Analysis of the loss in heat and acid resistance during germination of spores of Bacillus species,
Journal of Bacteriology, (05 2014): 1733. doi:

04/10/2012 37.00 Sonali Ghosh, Michelle Scotland, Peter Setlow. Levels of Germination Proteins in Dormant and Superdormant Spores of Bacillus subtilis ,
Journal of Bacteriology, (05 2012): 0. doi:

04/23/2014 65.00 Parvathimadhavi Devarakonda, Kristina Carlson, Andrew Davis, Kerry-Ann V. Stewart, Elizabeth Cammett, Patricia Pelczar Rossi, Barbara Setlow, Min Lu, Peter Setlow, Bing Hao, Kai Jin, Yunfeng Li, Sonali Ghosh. Structural and Functional Analysis of the GerD Spore Germination Protein of Bacillus Species,
Journal of Molecular Biology, (05 2014): 1995. doi: 10.1016/j.jmb.2014.02.004

04/27/2012 40.00 Daniel Paredes-Sabja, Mahfuzur R. Sarker. Interactions between Clostridium perfringens spores and Raw 264.7 macrophages,
Anaerobe, (02 2012): 148. doi: 10.1016/j.anaerobe.2011.12.019

04/27/2012 41.00 Pathima Udompijitkul, Daniel Paredes-Sabja, Mahfuzur R. Sarker. Inhibitory Effects of Nisin Against?Clostridium perfringens?Food Poisoning and Nonfood-Borne Isolates,
Journal of Food Science, (01 2012): 51. doi:

05/01/2011 13.00 Kong, Lingbo; Zhang, Pengfei; Wang, Guiwen; Yu, Jing; Setlow, Peter; Li, Yongqing. Characterization of bacterial spore germination using phase contrast and fluorescence microscopy, Raman spectroscopy and optical tweezers,
Nature Protocols, (04 2011): . doi:

05/01/2011 14.00 G. Wang, X. Yi, Y.-q. Li, P. Setlow. Germination of individual Bacillus subtilis spores with alterations in the GerD and SpoVA proteins, which are important in spore germination,
Journal of Bacteriology, (02 2011): . doi:

05/10/2010 2.00 J. Wei, I.M. Shah, S. Ghosh, J. Dworkin, D.G. Hoover, P. Setlow.. Superdormant spores of Bacillus species germinate normally with high pressure, peptidoglycan fragments and bryostatin. ,
Journal of Bacteriology, (01 2010): . doi:

05/10/2010 1.00 L. Kong, P. Zhang, P. Setlow, Y-q. Li. Characterization of bacterial spore germination using integrated phase contrast microscopy, Raman spectroscopy and optical tweezers. ,
Analytical Chemistry, (05 2010): . doi:

05/18/2011 15.00 G. Wang, X. Yi, Y.-q. Li, P. Setlow. Germination of individual Bacillus subtilis spores with alterations in the GerD and SpoVA proteins, which are important in spore germination,
Journal of Bacteriology, (02 2011): . doi:

05/25/2012 38.00 Kerry-Ann V. Stewart, Xuan Yi, Sonali Ghosh, Peter Setlow. Germination Protein Levels and Rates of Germination of Spores of Bacillus subtilis with Overexpressed or Deleted Genes Encoding Germination Proteins,
Journal of Bacteriology, (06 2012): 3156. doi:

06/05/2013 50.00 Yunfeng Li, Xuan Y. Butzin, Andrew Davis, Barbara Setlow, George Korza, Fatma Isik Ustok, Graham Christie, Peter Setlow, Bing Hao. Activity and regulation of various forms of CwlJ, SleB and YpeB proteins in degrading cortex peptidoglycan of spores of Bacillus species in vitro and during spore germination,
Journal of Bacteriology, (06 2013): 2530. doi:

06/05/2013 49.00 Keren Griffiths, Ann Cowan, Peter Setlow, Ji Yu, Jingqiao Zhang. Expression level of Bacillus subtilis germinant receptors determines the average rate but not the heterogeneityof spore germination ,
Journal of Bacteriology, (04 2013): 1735. doi:

06/09/2010 4.00 n.d. lamontagne. Tracking refractive and molecular changes during bacterial spore germination,
Analytical Chemistry, (06 2010): . doi:

06/13/2011 16.00 L. Kong, P. Zhang, G. Wang, J. Yu, P. Setlow, Y.-Q. Li.. Phase contrast microscopy, fluorescence microscopy, Raman spectroscopy and optical tweezers to characterize the germination of individual bacterial spores,
Nature Protocols, (04 2011): . doi:

06/13/2011 18.00 D. Paredes-Sabja, M.R. Sarker. Germination response of spores of the pathogenic bacterium Clostridium difficile to cultured human epithelial cells,
Anaerobe, (02 2011): . doi:

06/13/2011 17.00 G. Wang, P. Zhang, P. Setlow, Y.Q. Li. Kinetics of germination of wet heat-treated individual spores of Bacillus species as followed by Raman spectroscopy and differential interference contrast microscopy,
Applied and Environmental Microbiology, (03 2011): . doi:

06/15/2010 3.00 P. Zhang, L. Kong, P. Setlow, Y.Q. Li. Characterization of wet heat inactivation of single spores of Bacillus species by dual-trap Raman spectroscopy and elastic light scattering. ,
Applied and Environmental Microbiology, (01 2010): . doi:

06/15/2010 5.00 Xuan Yi, Peter Setlow. Studies of the commitment step in the germination of spores of Bacillus species, Journal of Bacteriology, (04 2010): . doi:

07/06/2010 6.00 P. Zhang, W. Garner, X. Yi, Ji Yu, Y-q. Li, P. Setlow. Factors affecting the variability in the time between addition of nutrient germinants and rapid DPA release during germination of spores of Bacillus species, Journal of Bacteriology, (07 2010): . doi:

07/08/2011 19.00 P. Zhang, L. Kong, G. Wang, P. Setlow, Y.Q. Li. Monitoring the wet-heat inactivation dynamics of single spores of Bacillus species by using Raman-tweezers, differential interference contrast microscopy, and nucleic acid dye fluorescence microscopy,
Applied and Environmental Microbiology, (05 2011): . doi:

07/10/2012 39.00 Arturo Ramirez-Peralta, Kerry-Ann V. Stewart, Stacy K. Thomas, Barbara Setlow, Zhan Chen, Yong-Qing Li, Peter Setlow. Effects of the SpoVT regulatory protein on the germination and germination protein levels of spores of Bacillus subtilis,
Journal of Bacteriology, (07 2012): 3417. doi:

07/14/2011 20.00 L. Kong, P. Zhang, J. Yu, P. Setlow, Y.Q. Li. Rapid confocal Raman imaging using a synchro multifoci-scan scheme for dynamic monitoring of single living cells,
Applied Physics Letters, (05 2011): . doi:

07/14/2011 21.00 L King, P. Zhang, J Yu, P Setlow, YQ Li. Rapid confocal Raman imaging using a synchro multifoci-scan scheme for dynamic monitoring of single living cells,
Applied Physics Letters, (05 2011): . doi:

07/18/2011 22.00 J Zhang, W Garner, P Setlow, J Yu. Quantitative analysis of spatial-temporal correlations during germination of spores of Bacillus species,
Journal of Bacteriology, (05 2011): . doi:

07/29/2011 23.00 X. Yi, C. Bond, M. R. Sarker, P. Setlow. Efficient Inhibition of Germination of Coat-Deficient Bacterial Spores by Multivalent Metal Cations, Including Terbium (Tb3+),
Applied and Environmental Microbiology, (06 2011): 0. doi: 10.1128/AEM.00577-11

07/29/2011 24.00 Y. Li, P. Catta, K.-A. V. Stewart, M. Dufner, P. Setlow, B. Hao. Structure-Based Functional Studies of the Effects of Amino Acid Substitutions in GerBC, the C Subunit of the Bacillus subtilis GerB Spore Germinant Receptor,
Journal of Bacteriology, (08 2011): 4143. doi: 10.1128/JB.05247-11

08/01/2012 43.00 J Barras-Carrasco, C Hernandez-Rocha, M Pizzaro-Guajardo, P Ibanez, SM Bueno, MR Sarker, AM Guzman, M Alvarez-Lobos, D Paredes-Sabja. Epidemic Clostridium difficile ribotype 027 in Chile,
Emerging Infectious isease, (08 2012): 1370. doi:

08/01/2013 51.00 Arturo Ramirez-Peralta, Srishti Gupta, Xuan Yi Butzin, Barbara Setlow, George Korza, Marco Antonio Leyva-Vazquez , Graham Christie, Peter Setlow. Identification of new proteins that modulate the germination of spores of Bacillus species,
Journal of Bacteriology, (07 2013): 3009. doi:

08/01/2013 55.00 Peter Setlow, Yong-qing Li, Lingbo Kong. Observation of dynamic germination of single bacterial spores using rapid Raman imaging,
Journal of Biomedical Optics, (01 2014): 11003. doi:

08/01/2013 54.00 Kerry-Ann V. Stewart, Peter Setlow. Numbers of individual nutrient germinant receptors and other germination proteins in spores of Bacillus subtilis,
Journal of Bacteriology, (08 2013): 3575. doi:

08/01/2013 53.00 Bjorn Traag, Arturo R. Peralta, Ann F. W. Erickson, Peter Setlow, Richard Losick. A novel RNA polymerase-binding protein controlling genes involved in spore germination in Bacillus subtilis ,
Molecular Microbiology, (07 2013): 113. doi:

08/02/2012 44.00 Lingbo Kong, Peter Setlow, Yong-qing Li. Analysis of the Raman spectra of Ca2+-dipicolinic acid alone and in the bacterial spore core in both aqueous and dehydrated environments,
The Analyst, (08 2012): 3683. doi: 10.1039/c2an35468c

08/26/2010 7.00 Y. Li, B. Setlow, P. Setlow, B. Hao. Crystal Structure of the GerBC Component of a Bacillus subtilis Spore Germinant Receptor,
Journal of Molecular Biology, (09 2010): . doi:

09/06/2013 56.00 Lingbo Kong, Peter Setlow, Yong-qing Li. Direct analysis of water content and movement in single dormant bacterial spores using confocal Raman microspectroscopy and Raman imaging,
Analytical Chemistry, (08 2013): 7094. doi:

09/11/2014 71.00 David Pan, George Korza, Florence E Feeherry, Abigail Perez-Valdespino, Yunefeng Li, Barbara Setlow, Sonali Ghosh, Christopher J Doona, Yong-qing Li, Bing Hao, Peter Setlow. Function of the SpoVAEa and SpoVAF proteins of Bacillus subtilis spores,
Journal of Bacteriology, (06 2014): 2077. doi:

09/11/2014 72.00 Christopher J. Doona, Sonali Ghosh, Florence F. Feeherry, Arturo Ramirez-Peralta, Yaoxing Huang, Haiqiang Chen, Peter Setlow. High pressure germination of Bacillus subtilis spores with alterations in levels and types of germination proteins ,
Journal of Applied Microbiology, (09 2014): 711. doi:

09/11/2014 75.00 AD White , Q Shao, P Setlow, YQ Li, S Jiang, M Luo. Chemical insights into dodecylamine spore lethal germination,
Chemical Science, (06 2014): 3320. doi:

09/11/2014 73.00 Pengfei Zhang, Jintao Liu, Xuan Yi, Peter Setlow, Yong-qing Li. Monitoring of Commitment, Blocking and Continuation of Nutrient Germination of Individual Bacillus subtilis Spores,
Journal of Bacteriology, (07 2014): 2443. doi:

09/19/2012 42.00 Mark J. Leggett, Gerald McDonnell, Stephen P. Denyer, Peter Setlow, Jean-Yves Maillard. Bacterial spore structures and their protective role in biocide resistance.,
Journal of Applied Microbiology, (09 2012): 485. doi: 10.1111/j.1365-2672.2012.05336.x

09/19/2012 45.00 Guiwen Wang, Daniel Paredes- Sabja, Mahfuzur R. Sarker, Calvert Green, Peter Setlow, Yong-qing Li. Effects of wet heat-treatment on the germination of individual spores of Clostridium perfringens,
Journal of Applied Microbiology, (10 2012): 824. doi: 10.1111/j.1365-2672.2012.05387.x

09/27/2014 76.00 M Plomp, AM Carroll, P Setlow, AJ Malkin. Architecture and assembly of the Bacillus subtilis spore coat,
PLoS ONE, (09 2014): 108560. doi:

10/07/2010 8.00 P. Zhang, L. Kong, P. Setlow, Y.Q. Li. Multiple-trap laser tweezers Raman spectroscopy for simultaneous monitoring of the biological dynamics of multiple individual cells ,
Optics Express, (10 2010): . doi:

10/09/2014 74.00 J Liang, P Zhang, P Setlow, YQ Li. High precision fitting measurements of the kinetics of size changes during germination of individual Bacillus spores,
Applied and Environmental Microbiology, (08 2014): 4606. doi:

11/13/2011 25.00 P. Zhang, D. Paredes-Sabja, G. Wang, C. Green, P. Setlow, M.R. Sarker, Y.-Q. Li. Analysis of the germination of individual Clostridium perfringens spores and its heterogeneity,
Journal of Applied Microbiology, (11 2011): 0. doi: 10.1111/j.1365-2672.2011.05135.x

12/11/2014 79.00 Pathima Udompijitkul, Maryam Alnoman, Saeed Banawas, Daniel Paredes-Sabja, Mahfuzur R. Sarker. New amino acid germinants for spores of the enterotoxigenic Clostridium perfringens type A isolates,
Food Microbiology, (12 2014): 24. doi: 10.1016/j.fm.2014.04.011

12/24/2014 77.00 Troiano AJ, Zhang J, Cowan AE, Yu J, Setlow P. Analysis of the dynamics of a Bacillus subtilis spore germination protein complex during spore germination and outgrowth,
Journal of Bacteriology, (01 2015): 252. doi:

12/26/2011 26.00 Jose-Luis Sanchez-Salas, Barbara Setlow, Pengfei Zhang, Yong-qing Li, Peter Setlow. Maturation of released spores is necessary for acquistion of full spore heat resistance during Bacillus subtilis sporulation,
Applied and Environmental Microbiology, (10 2011): 0. doi:

12/26/2011	27.00	J. Liu, X. Yi, J. R. Faeder, P. Setlow. Synergism between Different Germinant Receptors in the Germination of Bacillus subtilis Spores, Journal of Bacteriology, (07 2011): 0. doi: 10.1128/JB.05343-11
12/26/2011	28.00	Keren K. Griffiths, Jingqiao Zhang, Ann E. Cowan, Ji Yu, Peter Setlow. Germination proteins in the inner membrane of dormant bacillus subtilis spores colocalize in a discrete cluster, Molecular Microbiology, (08 2011): 0. doi:
12/26/2011	29.00	Lingbo Kong, Pengfei Zhang, Peter Setlow, Yong-qing Li. Multifocus confocal Raman spectroscopy for rapid single-particle analysis, Journal of Biomedical Optics, (10 2011): 0. doi:
12/26/2011	30.00	daniel Paredes-Sabja, Nahid Sarker, Mahfuzur R. Sarker. Clostridium perfringens tpeL is expressed during sporulation, Microbial pathogenesis, (11 2011): 0. doi:
TOTAL:	**74**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
01/24/2012	33.00	Pengfei Zhang, Stacy Thomas, Yong-qing Li, Peter Setlow. Effects of Cortex Peptidoglycan Structure and Cortex Hydrolysis on the Kinetics of Ca2+-Dipicolinic Acid Release During Bacillus subtilis Spore Germination, Journal of Bacteriology, (02 2012): 0. doi:
02/19/2014	57.00	R. Losick , B. A. Traag, A. Pugliese, B. Setlow, P. Setlow. A conserved ClpP-like protease involved in spore outgrowth in Bacillus subtilis, Molecular Microbiology, (10 2013): 160. doi:
10/10/2012	46.00	Xuan Y. Butzin, Anthony J. Troiano, William H. Coleman, Keren K. Griffiths, Christopher J. Doona, Florence E. Feeherry, Guiwen Wang, Yong-qing Li, Peter Setlow. Analysis of the effects of a gerP mutation on the germination of spores of Bacillus subtilis, Journal of Bacteriology, (11 2012): 5749. doi:
TOTAL:	**3**	

Number of Papers published in non peer-reviewed journals:

(c) Presentations

See full list of presentations in Attachment 3

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
John Sekar	1.00	
Robert Sheehan	0.40	
FTE Equivalent:	**1.40**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Shiwei Wang	0.85
Prabhat Talukdar	0.30
Abigail Perez-Valdespino	1.00
FTE Equivalent:	**2.15**
Total Number:	**3**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Peter Setlow	0.35	
Bing Hao	0.10	
James Faeder	0.05	
Yong-qing Li	0.50	
Barbara Setlow	0.20	
FTE Equivalent:	**1.20**	
Total Number:	**5**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Jing Yu	0.75
Yunefeng Li	0.20
FTE Equivalent:	**0.95**
Total Number:	**2**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment 1

Technology Transfer

There has been continued interaction and collaboration between the labs of Christopher J. Doona at the Natick Soldier Center and the labs of P. Setlow and Y.-Q. Li. This collaborative work has been looking primarily at factors that alter high pressure germination of spores of Bacillus species. In the current reporting period, this work has resulted in the publication of one manuscript on effects of various germination proteins on high pressure germination of spores. In addition, a second manuscript that includes studies on the effects of heat activation on high pressure germination of spores is currently being revised prior to resubmission.

Table of Contents

Attachment 1

Scientific Progress

a) Progress since August 1, 2014

Progress was significant in the last ~ 4 months of grant support, as 7 papers were published, 7 papers are in press, and three are in review. In addition, several manuscripts are in preparation. Major findings in these last ~ four months are as follows.

A) Li lab, with Setlow lab. We have made the striking observation that spores of *Bacillus* species given a short pulse of a nutrient germinant after which the germinant is washed away, exhibit much higher levels of germination after a second germinant pulse. This "memory" of the first pulse decays with longer times or higher temperatures in the period between germinant pulses, and is stabilized at low temperatures. Since the memory acquired can be reversed, it is acquired before the time, T_c, when spores become irreversibly committed to germinate, and many min before spores begin rapid dipicolinic acid (DPA) release in spore germination. This memory of an initial germinant pulse can be generated by germinants that are recognized by any nutrient germinant receptor (GR) and the memory can be accessed upon stimulation of any GR. This memory is also seen in the germination of spores of several *Bacillus* species! Work is in progress to determine if this memory is unique to GR-dependent germination, or can also be observed in germination stimulated by non-GR-dependent germinants. This latter experimental work should allow determination of where this memory is stored – is it in GRs, or in some common downstream germination component such as the dipicolinic acid (DPA) release apparatus involving a channel composed of SpoVA proteins.

B) Li lab with Setlow lab. When exposed to nutrient or non-nutrient germinants, individual *Bacillus* spores can return to life through germination followed by outgrowth. Laser tweezers Raman spectroscopy, and either differential interference contrast or phase contrast microscopy were used to analyze the slow DPA leakage (normally ~20% of spore DPA) from individual spores that takes place prior to the lag time, T_{lag}, when spores begin rapid release of remaining DPA. Major conclusions from this work with *Bacillus subtilis* spores were: 1) slow DPA leakage from wild-type spores germinating with nutrients did not begin immediately after nutrient exposure, but only at a later heterogeneous time T_1; 2) the period of slow DPA leakage ($\Delta T_{leakage} = T_{lag} - T_1$) was heterogeneous among individual spores, although the amount of DPA released in this period was relatively constant; 3) increases in germination temperature significantly decreased T_1 times, but increased values of $\Delta T_{leakage}$; 4) upon germination with L-valine for 10 min followed by addition of D-alanine to block further germination, all germinated spores had T_1 times less than 10 min, suggesting that T_1 is the time, T_c, when spores become committed to

germinate; 5) elevated levels of SpoVA proteins involved in DPA movement in spore germination decreased T_1 and T_{lag} times, but not the amount of DPA released in $\Delta T_{leakage}$; 6) lack of the cortex-lytic enzyme CwlJ increased DPA leakage during germination due to longer $\Delta T_{leakage}$ times in which more DPA was released; and 6) there was slow DPA leakage early in germination of *B. subtilis* spores by the non-nutrients CaDPA and dodecylamine and in nutrient germination of *Bacillus cereus* and *Bacillus megaterium* spores. Overall, these findings have identified and characterized a new early event in *Bacillus* spore germination.

C) Faeder lab, with Li and Setlow labs. We have developed a mathematical model of bacterial spore germination that accounts for heterogeneity in both T_{lag} and commitment times. The model is built from three main mathematical components: *a receptor distribution function* characterizing the probability of a given spore having a particular number of GRs, a *receptor activation function* that determines what fraction of a spore's GRs are active given a set of external inputs (e.g., nutrient concentrations), and a *germination kinetics function* that specifies the number of active GRs a spore must contain in order to germinate by a given time *t*. This model is then used to predict the fraction of spores germinating as a function of time and germinant concentration. The parameters of the model can be obtained by fitting to experimental data on germination kinetics either from *populations* or *individual* spores. The model can also be used to fit commitment data, in which case the germination kinetics function describes the number of active GRs a spore must contain in order to *commit* by time *t*. Germination kinetics following the quenching of germination in commitment experiments can also be used to determine how the time between commitment and T_{lag} varies with GR number and commitment time. Given germination data for combinations of germinants acting through different GRs, the model can be used to select or rule out plausible mechanisms of signal combination. We have found that the observed synergy between the GerA and GerB* GRs in FB10 spores can be accounted for by a model in which a common signaling intermediate accumulates at a rate proportion to the sum of the number of active GRs, but not by a model in which these GRs generate non-overlapping signals. The model can recapitulate a wide range of data generated in both wild-type and mutant strains for single and double nutrient germination, but underestimates DPA release kinetics in FB10 spore *populations* stimulated to germinate by L-asparagine. Publication of the mathematical model has been held up in order to reconcile these differences. Recent measurements from the Li lab (see **aB)** above) on *individual* spores indicate that upon exposure to germinants, spores can release DPA slowly before undergoing the rapid DPA loss characteristic of germination. This DPA leakage is not accounted for by the model outlined above, possibly leading to the observed discrepancies. DPA released in the T_{lag} period makes it appear as if spores are germinating at times when they actually are not, highlighting the importance of using single-spore data for comparison with models when mechanistic inference is the goal. Fitting of previous and newly acquired germination kinetics data from the Li lab with both single and double nutrient stimulation is currently underway and appears to support previous conclusions about the mechanism of synergy. This model is also in principle capable of accounting for the memory effects described above in **aA)** and can also be used to predict the kinetics of germination triggered by high hydrostatic pressure. We expect to submit a manuscript describing the model and its application to this data in early 2015, as well as to apply the model to memory in both germinant-induced and HP germination in subsequent manuscripts.

D) Setlow lab, with Yu and Cowan labs. We have been able to use *B. subtilis* spores lacking most coat proteins and that also carry a fluorescent fusion to a germination protein as well as a germination gene fused to the *lacZ* gene for β-galactosidase to follow germinosome assembly during spore formation. To date this work has shown that germinosome assembly takes place essentially in parallel with synthesis of germination protein components of the germinosome, and well before developing spores accumulate DPA. In addition, assembly of two different germinosome components takes place in parallel during sporulation. These results indicate that GRs and GerD, the major and perhaps only germinosome components, can self-assemble in the developing spore's inner membrane, and require no special changes in the IM for this assembly.

E) Setlow lab*. This work was undertaken to obtain information on levels of metabolism in dormant spores of *Bacillus* species incubated for weeks at physiological temperatures. Spores of *Bacillus megaterium* and *B, subtilis* strains were harvested shortly after release from sporangia, incubated under various conditions and dormant spore metabolism monitored by ^{31}P-NMR of molecules including 3-phosphoglyceric acid (3PGA) and ribonucleotides. Incubation for up to 30 d at 4°, 37° or 50°C in water, at 37° or 50°C in buffer to raise spore core pH from ~ 6.3 to 7.8, or at 4°C in spent sporulation medium caused no significant changes in ribonucleotide or 3PGA levels. Stage I germinated spores of *B. megaterium* that had slightly increased core water content and a core pH of 7.8 also did not degrade 3PGA and accumulated no ribonucleotides, including ATP, during incubation for 8 d at 37°C in buffered saline. In contrast, spores incubated for up to 30 d at 37 or 50°C in spent sporulation medium degraded significant amounts of 3PGA, and accumulated ribonucleotides, indicative of RNA degradation, and these processes were increased in *B. megaterium* spores with a core pH of ~7.8. However, no ATP was accumulated in these spores. These data indicate that spores of *Bacillus* species stored in water or buffer at low or high temperatures exhibited minimal if any metabolism of endogenous compounds, even if spore core pH is 7.8 and core water content is increased somewhat. However, there was some metabolism in spores stored in spent sporulation medium.
*This work was supported primarily by a STIR award to P. Setlow, but some MURI funds were also used for this project.

b) Scientific Progress and Major Findings During the Entire Period of MURI Support

There has been tremendous progress throughout the entire period of this MURI, as there have been ~ 85 papers published with MURI support. These are primarily research papers, but include ~ 8 review articles in either journals or book chapters. In addition, there are four additional review articles in press and three research papers in press, with an additional 3 research papers in review. We also are confident that there will be > 3 more research papers submitted in the next few months based on MURI supported work. In total, it appears most likely that this MURI project will lead to more than 100 publications, primarily on various aspects of bacterial spore germination and its heterogeneity, as well as some papers on other aspects of bacterial spores and their formation.

Major Scientific Accomplishments in the entire MURI period are summarized below.
More detailed descriptions of specific research findings in the first 5 years of MURI support can be found in the Interim Progress Reports for each of the preceding years.

A) Structural Biology: Prior to the MURI award, the high-resolution structures of no spore germination proteins had been determined. However, we determined high-resolution structures by X-ray crystallography of: 1) the GerD germination protein; 2) a C-subunit of a germinant receptor (GR) subunit; 3) a GR A-subunit; and 4) the SleB *Bacillus* cortex-lytic enzyme. The SleB structure allowed identification of the enzyme's catalytic glutamate residue, and further allowed modeling of the structure of the other *Bacillus* cortex-lytic enzyme, CwlJ. This latter modeling also identified CwlJ's likely catalytic glutamate, and the essentiality of this glutamate residue was confirmed by site directed mutagenesis. The GerD, and GR A and C subunit structures were novel ones, but structure-function studies by appropriate site directed mutagenesis work with analyses of protein function *in vivo* in spore germination has allowed some assessment of how these proteins function in the overall spore germination process. In addition, our *in silico* analysis of the structure of the SpoVAD protein thought to be involved in DPA movement into and out of spores suggested that this protein might bind DPA. Indeed, we found that SpoVAD does bind the physiological DPA isomer specifically, and in a highly conserved DPA binding pocket. This work has provided some of the strong direct evidence that SpoVA proteins are involved directly in DPA movement in the processes of sporulation and germination. Further evidence of a specific role for at least one SpoVA protein specifically in DPA release in spore germination was the demonstration that spores lacking the SpoVAEa protein accumulated DPA normally in sporulation, but DPA release in GR-dependent germination was slowed > 3-fold.

B) Superdormant (SD) Spores: Spores that germinate very, very slowly and are termed SD, are the bane of the food industry, as such spores preclude using an initial germination step followed by mild killing regimens for spore removal. We were able to isolate and characterize spores that were SD for germination with GR-dependent germinants as a small percentage of spores in populations of spores of several *Bacillus* species. These spores germinated extremely slowly with the nutrient germinant used to isolate them, and even moderately slowly with other nutrient germinants, but normally with GR-independent germinants. Using antisera prepared against GerD and various GR subunits from *B. subtilis* we showed that levels of subunits of the GR recognizing the germinant used to isolate SD spores were very low in SD spores, while levels of other GR subunits were only slightly lower than normal and levels of GerD and SpoVA proteins were normal. In addition, isolated SD spores that were resporulated gave rise to spores that germinated essentially identically to the initial spore population. Thus SD spores are not formed due to mutation, but to some phenotypic effect, perhaps due to the normal wide distribution of the numbers of GRs in spore populations due to stochastic effects. This work further motivated us to examine factors that might influence levels of GRs in spores, and thus spores' rates of germination, as described in **bC)** below. In other work we also isolated spores SD for germination with the 1:1 chelate of Ca and DPA (CaDPA), a GR-independent germinant that triggers germination by activating the cortex-lytic enzyme CwlJ. These particular SD spores were found to have low levels of CwlJ, probably because their coats had been damaged and CwlJ lost.

C) Factors Affecting Levels of GRs in Spores: We used quantitative western blot analyses to determine levels of germination proteins in *B. subtilis* spores prepared under various conditions, and with and without various mutations. This work showed that: 1) spores prepared in richer media had higher GR levels and germinated faster; 2) spores of a strain lacking the SpoVT

regulatory protein that binds to DNA had elevated GR levels and germinated faster; and 3) spores of a strain lacking the regulatory protein YlyA that associates with RNA polymerase had lower GR subunit levels and germinated more slowly. Presumably the precise levels of SpoVT and YlyA in developing spores during sporulation, as well as presumably other factors that respond in some fashion to media richness all combine to determine GR levels in a spore. This combination of multiple regulatory factors present at low levels, as well as the normally low levels of GR subunits (~ 500 molecules/spore) combine with stochastic effects to give a distribution of spores in populations with widely variable GR numbers/spore, and thus different rates of spore germination in spore populations. This wide variation in GR numbers in spores throughout a spore population is presumably an example of what is termed "bet-hedging", ensuring that a whole spore population does not germinate at once, hence protecting the survival of the population against a drastic deleterious change in the poplation's environment shortly after initiating germination.

D) Early Events in Spore Germination: A combination of techniques has been used to examine events early in spore germination, analyzing multiple individual spores as well as spore populations. This work has shown that following addition of a nutrient germinant there is a period where nothing obvious is happening although spores can reversibly acquire "memory" of a germinant stimulus, such that given a later germinant stimulus they can respond much more readily than to the first stimulus. This period when only reversible events take place is longer in spores with low GR levels, or with low germinant concentrations and in spores without heat activation. This period ends at commitment when a spore is irreversibly committed to germinate even if the germinant is removed. The time of commitment, T_c, appears to coincide with the time $T_{leakage}$, when DPA begins to leak slowly from the spore. This latter change is most likely because there is a drastic change in the spore's inner membrane (IM) permeability properties at this time, either generally or because of a partial opening of the SpoVA protein channels for DPA in the IM. This DPA leakage results in slow release of ~ 20% of spores' DPA in ~ 5-15 min, and when the leakage ends at a time termed T_{lag}, all remaining DPA is released in 1-2 min, ending at $T_{release}$. $T_{release}$ is then followed by core swelling due to spore cortex peptidoglycan lysis that takes ~ 5-20 min and ends at T_{lysis}. This order of events in germination has been studied best in *B. subtilis*, but also has been seen in the germination of spores of several other *Bacillus* species, *Geobacillus stearothermophilus* and *Clostridium perfringens*. These kinetics have been studied best with GR-dependent germinants, but where it has been studied, are also seen in GR-independent germination as well.

E) Germination of *C. perfringens* Spores: Work examining the mechanisms and kinetics of *C. perfringens* spores has given a number of results that are similar to those with spores of *Bacillus* species. In particular: 1) germination kinetics appear to exhibit the same features as described in **aD)** above for *Bacillus* species, although spore memory has not yet been examined with *Clostridium* spores; 2) at least one *C. perfringens* GR C subunit is in the spore's inner membrane and has a similar topology in the IM to *B. subtilis* GR C-subunits; and 3) the level of this GR C subunit in *C. perfringens* spores is similar to that in spores of *Bacillus* species.

F) Location of Germination Proteins in *Bacillus* Spores: Using spores of *B. subtilis*, we have shown that all GR subunits and GerD are present in spores' IM. Even more interesting is that almost always all these proteins are located together in a small (~ 90 nm diameter) cluster in the

IM. Formation of this cluster, termed the germinosome, absolutely requires GerD, which may form an oligomeric scaffold for GR assembly as we have identified a dominant negative mutation in GerD. The GerD protein also forms a cluster in spores' IM in the absence of all GRs, and also to some degree when GerD is expressed in growing cells. The germinosome assembles in sporulation approximately in parallel with synthesis of the germinosome components and disperses only very slowly during spore germination and outgrowth. Since spores lacking GerD germinate very poorly with GR-dependent germinants, it seems highly likely that germinosome assembly is crucial for rapid GR-dependent spore germination. In contrast to GR subunits and GerD, the SpoVA proteins that are involved in DPA movement into and out of spores in sporulation and germination, respectively, appear to be dispersed throughout the IM in spores.

G) Methodology for Examining the Germination of Individual Spores: Beginning from the ability to examine the germination of one spore at a time by either DIC spectroscopy or Raman spectroscopy of spore DPA, we have made this technology much more robust. As a consequence, we have developed the ability to examine simultaneously events during the incubation of spores at temperatures from 15- to ~ 90°C and with or without germinants or other chemicals, and with changes in solution composition during incubation possible. Events in spores can be followed not only by Raman spectroscopy and DIC microscopy, but also by fluorescence or phase contrast microscopy and much of this can be done in a hands-free manner, allowing the collection of large amounts of data from multiple individual spores simultaneously. These technological advances have also been coupled to faster and faster measurement times to allow analysis of movement of D_2O into and out of spores, size changes during spore germination and the state of DPA in spores. In sum this work has provided a whole series of new ways to rapidly examine spore behavior, in particular the extreme phenotypic heterogeneity of spore responses to germinants or extreme conditions that is often obscured in analyses of the behavior of spore populations.

H**) Factors that Affect the Kinetics of Spore Germination:** Along with the improved methodology for monitoring the germination of multiple individual spores, we also have developed a simple assay in multi-well format for measuring germination of spore populations. This assay uses an automated multi-well fluorometric plate reader to measure DPA release during spore germination by adding low levels of Tb^{+3} to germination solutions and monitoring Tb-DPA fluorescence. This assay system is simple and avoids the false positive results that can be a problem when spore germination is monitored by measuring optical density at 600 nm. This multi-well fluorometric assay has now been adopted for use by others in the spore field. In our labs, it has allowed determination of precise kinetics of germination of populations of spores of various genotypes and under a wide variety of conditions. Advances from the use of this assay as well as analyses of the germination of multiple individual spores have included: 1) demonstration that spore populations of *B. subtilis* and *C. perfringens* killed 80-90% by heat, H_2O_2 or hypochlorite still germinate 60-75%, but values for T_{lag}, $T_{release}$ and T_{lysis} are increased greatly; 2) alterations in *B. subtilis* spore cortex peptidoglycan structure can greatly influence spore germination rates, primarily T_{lag} values, although in some cases T_{lysis} values also, while loss of CwlJ results in increases in $\Delta T_{release}$ values ($T_{release}$-T_{lag}) up to 10-fold; 3) small proteins encoded by genes within or just upstream of operons encoding *B. subtilis* or *B. megaterium* GRs can either positively or negatively influence germination by this GR; 4) overexpression of one

GR can increase germination by that GR and can greatly reduce germination by GRs that are not over-expressed, although levels of the latter GR(s) are not lowered; 5) a *gerP* mutation greatly increases T_{lag} times but not $\Delta T_{release}$ times, and unlike wild-type spores for which maximal germination rates were obtained with ~ 0.5 mM L-alanine, with *gerP* spores this required almost 1 M L-alanine; since *gerP* spores' germination with a high hydrostatic pressure (HP) of 150 megaPascals (MPa) that activates GRs was essentially identical to that of wild-type spores, it appears most likely that *gerP* spores are defective in germinant access to GRs; and 6) the massive amount of germination kinetic data for both individual spores and populations, as well as average numbers of GRs/spore is being used as described in **aC)** above to generate a mathematical model describing spore germination via various GRs.

I) Spore Germination by HP: Germination of individual *B. subtilis* spores by a HP of 150 MPa that is thought to activate GRs was studied in a diamond anvil cell. This work showed that this HP germination of individual spores exhibited the same kinetic features of nutrient germination via GRs, including $T_{leakage}$, T_{lag}, $T_{release}$ and T_{lysis} times. In addition, even prior to $T_{leakage}$, these HP-treated spores exhibited a reversible activation for subsequent germination, analogous to what we saw in the memory generated by a short nutrient germinant pulse that potentiated germination upon a second short nutrient germinant pulse. HP germination of populations of spores of various genotypes also showed that the two main factors in germination at 150 MPa were GR levels, since germination rates increased as GR levels increased, while loss of GerD or a dominant negative GerD variant greatly decreased 150 MPa spore germination. In contrast, germination with a HP of 500 MPa was not affected by changes in GR levels of GerD. However, germination at both 150 and 550 MPa was increased markedly by a heat activation treatment prior to application of HP.

Attachment 2

Papers - Aug 1, 2014-Dec 31, 2014 (and beyond)

Manuscripts: a) Published (7); b) In press (7); or c) Submitted (3) (names in bold are MURI team members)

a) Published

1. Doona, C.J., S. Ghosh, F.F. Feeherry, A. Ramirez-Peralta, Y. Huang, H. Chen, and **P. Setlow**. 2014. High pressure germination of *Bacillus subtilis* spores with alterations in levels and types of germination proteins. J. Appl. Microbiol. **117**:711-20.
2. Udompijitkul, P., M. Alnoman, S. Banawas, D. Paredes-Sabjam and **M.R. Sarker**. 2014. New amino acid germinants for spores of the enterotoxigenic *Clostridium perfringens* type A isolates. Food. Microbiol. **44**:24-33.
3. Zhang, P. J. Liang, X. Yi, **P. Setlow**, and **Y.q, Li**. 2014. Monitoring of commitment, blocking and continuation of nutrient germination of individual *Bacillus subtilis* spores. J. Bacteriol. **196**:2443-2454.
4. Liang, J., P. Zhang, **P. Setlow** and **Y.q. Li**. 2014. High precision fitting measurements of the kinetics of size changes during germination of individual *Bacillus* spores. Appl. Environ. Microbiol. **80**:4606-4615.
5. Luo, M., A.D. White, Q. Shao, **P. Setlow**, **Y.-q. Li**, and S. Jiang. 2014. Chemical insights into dodecylamine spore lethal germination. Chem. Science **5**, 3320-3324.
6. Plomp, M., A.M. Carroll, **P. Setlow**, and A. J. Malkin. 2014. Architecture and assembly of the *Bacillus subtilis* spore coat. PloS One **9**:e108560.
7. Troiano, A.J., J. Zhang, **A.E. Cowan**, **J. Yu**, and **P. Setlow**. 2015. Analysis of the dynamics of a *Bacillus subtilis* spore germination protein complex during spore germination and outgrowth. J. Bacteriol. **197**:240-251 (Selected as a Spotlight paper in this issue of Journal of Bacteriology).

b) In press

1) P. K. Talukdar, V. Olguín-Araneda3, M. Alnoman, D. Paredes-Sabja, and **M. R. Sarker**. 2014. Updates on the sporulation process in *Clostridium* species. Research in Microbiology. In Press.
2. **Setlow, P.** 2015. Spore resistance properties, in press. *In* Eichenberger P, Driks A (ed), The Bacterial Spore. ASM Press, Washington, DC.
3. Akhtar, S., **M. R. Sarker**, K. Jabeen, A. Qamar,. and A. Sattar. 2014. Antimicrobial resistance in *Salmonella enterica* serovar Typhi and Paratyphi in South Asia - Current status, issues and prospects. Crit. Rev. Microbiol. In press.
4. Olguín-Araneda, V., S. Banawas, **M.R. Sarker**, D. Paredes-Sabja. 2014. Recent advances in germination of *Clostridium* spores. Res. Microbiol. in press.
5. Ghosh, S., G. Korza, M. Maciejewski, and **P. Setlow**. 2015. Analysis of metabolism in dormant spores of *Bacillus* species by ^{31}P-NMR of low molecular weight compounds. J. Bacteriol. In press.
6. Wang, S., **P. Setlow**, and **Y-q. Li**. 2015. Slow leakage of Ca-dipicolinic acid from individual *Bacillus* spores during initiation of spore germination. J. Bacteriol. In press.
7) M. Alnoman, P. Udompijitkul, D. Paredes-Sabja, and **M. R. Sarker**. 2014. The inhibitory effects of sorbate and benzoate against *Clostridium perfringens* type A isolates. Food Microbiol. In Press.

c) Submitted

1. Cruz-Mora, J., A. Pérez-Valdespino, S. Gupta, N. Withange, R. Kuwana, H Takamatsu, G. Christie, and **P. Setlow**. 2014. The GerW protein is not involved in the germination of spores of *Bacillus* species. PLoS One. Submitted.
2. Yasugi, M., H. Hoshi, D. Okuzaki, P.K. Talukdar, K. Kondo, S. Yamamoto, Y. Kamata, **M.R. Sarker**, and M. Miyake. 2014. Bile acids accelerate sporulation via Spo0A activation in *Clostridium perfringens*. PLoS One, submitted.
3. Luu, S., Jose Cruz-Mora, B. Setlow, F.E. Feeherry, C.J. Doona, and **P. Setlow**. 2014. The effects of heat activation on nutrient and high-pressure germination of spores of *Bacillus subtilis* with and without various germination proteins. Appl. Environ. Microbiol. Submitted.

Attachment 3

Presentations – Aug 1, 2014- Dec 31, 2014 (names of MURI team members are in bold)

1. **P. Setlow**. When the Sleepers Wake: The Germination of Spores of *Bacillus* Species. Louisiana Tech University, Ruston, LA, Dec 8, 2014.
2. **P. Setlow**. Killing of Spores of Bacillus Species in a High Temperature Gas Environment. DTRA Research Review, August 1, 2014, Springfield, VA.
3. **Yong-qing Li**, Monitoring and analysis of single cell dynamics using confocal Raman imaging and ultralow frequency Raman micro-spectroscopy, The XXIV International Conference on Raman Spectroscopy at Jena, Germany, August 10-15, 2014. Invited talk.
4. **Yong-qing Li**, (a) The discovery of cellular memory of response to nutrient exposures. (b) Optical pulling, trapping and identification of airborne absorbing particles and bioaerosols. Invited presentations at Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences, Shanghai, China, November 19, 2014.